# A comparison of donor lymphocyte infusions or imatinib mesylate for patients with chronic myelogenous leukemia who have relapsed after allogeneic stem cell transplantation

Martin Weisser
Johanna Tischer
Susanne Schnittger
Claudia Schoch
Georg Ledderose
Hans Jochem Kolb

Imatinib mesylate is highly effective in relapsed chronic myelogenous leukemia (CML) after allogeneic hematopoetic stem cell transplantation (HSCT). However, it is unknown whether imatinib produces durable molecular remissions. The outcome of CML patients transplanted at our center who had received only imatinib for relapse after HSCT was compared with that of patients treated with donor lymphocyte infusions (DLI). Imatinib therapy resulted in a higher incidence of relapse and inferior leukemia-free survival ($p$=0.006 and $p$=0.016, respectively). These data suggest that imatinib alone probably does not cure relapse after HSCT.

Key words: donor lymphocyte infusion, imatinib, chronic myelogenous leukemia, allogeneic stem cell transplantation, relapse.

Haematologica 2006; 91:663-666



*From the hematopoetic cell transplantation, Department of Medicine III, University of Munich, Klinikum Grosshadern, Ludwig Maximilians University of Munich and GSF-National Research Center for Environment and Health, Munich, Germany (MW, JT, GL, HJK); Munich Leukemia Laboratory, Munich, Germany (SS, CS).*

*Correspondence:*
*Martin Weisser, Department of Internal Medicine III, University of Munich, Klinikum Grosshadern, Marchioninistr. 15, 81377 Munich, Germany.*
*E-mail: martin.weisser@med.uni-muenchen.de*

At present, donor lymphocyte infusion (DLI) is the standard therapy for patients with chronic myelogenous leukemia (CML) relapsing after allogeneic hematopoetic stem cell transplantation (HSCT). DLI has proven to restore full donor chimerism and produce long-term complete molecular genetic remissions.[1-3] However, possible side effects, such as graft-versus-host disease (GvHD) and myelosuppression lead to a considerable amount of treatment-related morbidity and mortality. Imatinib mesylate has shown remarkable short-term efficacy in the treatment of CML combined with low toxicity.[4-8] However, the follow-up of patients treated with imatinib is still rather short and it remains unclear whether this drug has the potential to create durable molecular remissions, maintain these responses after discontinuation of the drug, and thus lead to definite cure of relapse. We, therefore, retrospectively analyzed the long-term outcome of CML patients who had received imatinib alone for therapy of relapse after allogeneic HSCT at our center. The results were compared to the outcome of patients who were treated with DLI as relapse therapy.

## Design and Methods

### Patients

We evaluated patients with *BCR-ABL* positive CML who had undergone allogeneic HSCT in chronic or accelerated phase and who had relapsed after the transplant. Patients had received either DLI or imatinib for treatment of relapse. Imatinib was given in cases in which DLI was difficult to obtain, explaining the larger proportion of unrelated donors in the imatinib group. Thirty-one CML patients were evaluable, of whom 21 were treated with DLI and ten with imatinib. The median follow-up time was 1230 days (range 180-3350 days). Imatinib was administered at a dose of 400 mg-800 mg/day depending on response. Imatinib was escalated in 200 mg steps if there was an satisfactory response. An unsatisfactory response was defined as stable polymerase chain reaction (PCR) ratios in three consecutive independent PCR samples within one log of the baseline level over a period of 3 months, rising PCR ratios by at least one log of the baseline level in two consecutive samples, or signs of hematologic progress. The median duration of imatinib therapy was 365 days (range 45-1530). The median number of DLI was 1.5 per patient (range 1-3). The CD3 cell concentration ranged from $5\times10^5$-$1\times10^8$/kg. The initial cell dose was aimed to be $1\times10^6$/kg for unrelated donors and $1\times10^7$/kg for sibling donors. Lower initial cell doses were administered to patients with a history of severe acute GvHD after allogeneic HSCT. Patients received up to three infusions of donor lymphocytes, with escalating cell doses, at intervals of 4-6 weeks provided there were no clinical signs of GvHD. The patients' characteristics are shown in Table 1.

### Molecular cytogenetic and molecular genetic investigations

In order to evaluate response to therapy, routine fluorescent *in situ* hybridization (FISH) analysis for *BCR-ABL*-rearrangement

**Table 1.** Patients' characteristics.

| | *Imatinib (n=10)* | *DLI (n=21)* | *p value* |
|---|---|---|---|
| Median age (years) | 33 (26-57) | 37 (22-59) | 0.452 |
| Sex | 6 m/4 f | 13 m/8 f | 1.00 |
| Stage at HSCT | | | 0.652 |
| Chronic phase | 7 | 17 | |
| Accelerated phase | 3 | 4 | |
| Donor | | | 0.252 |
| Sibling | 3 | 12 | |
| Unrelated | 7 | 9 | |
| GvHD-prophylaxis | n=10 | n=21 | |
| CsA/MTX | | | |
| Median time to 1st relapse (days) | 849 (104-6205) | 692 (364-2323) | 0.704 |
| Type of relapse | | | 1.00 |
| Cytogenetic | 9 | 14 | |
| Hematologic | 1 | 7 | |
| Median time to 2nd relapse (days) | 431 (45-1460) | 730 (122-991) | 0.656 |

*CsA: cyclosporine A; MTX: methotrexate.*

was performed in all cases on interphase nuclei from bone marrow smears. The background level for FISH analysis was 5%. For further details of the techniques used, refer to the descriptions by Schoch *et al.*[9]

### Nucleic acid isolation

Mononuclear cells were obtained by Ficoll density gradient centrifugation. For each sample mRNA from $5\times10^6$ human cells was isolated according to the Magna Pure LC mRNA protocol for human cells (Roche Diagnostics, Mannheim, Germany) and eluted in 30 μL $H_2O$.

### cDNA synthesis

Random primed cDNA synthesis was performed with Superscript II (Invitrogen, San Diego, Ca, USA) on diagnostic samples with 5 μL of mRNA corresponding to approximately $0.8\times10^6$ cells in a 50 μL reaction.

### Quantitative real-time PCR

Quantitative PCR was performed using the LightCycler® System (Roche Diagnostics, Mannheim, Germany) applying hybridization probes as the detection format. The PCR conditions, primers and hybridization probes used to amplify and quantify the *BCR-ABL* fusion transcripts were as described by Emig *et al.*[10] The expression of *BCR-ABL* was normalized against the expression of the control gene *ABL* to adjust for variations in RNA quality and efficiencies of cDNA synthesis. The expression ratios are given as: 100×*BCR-ABL/ABL*. *ABL* expression was quantified according to Emig *et al.*[10] In addition, qualitative nested RT-PCR was performed as reported by Maurer *et al.*[11]

### Definition of relapse to imatinib or DLI therapy

Relapse was defined as: (i) primary hematologic/cytogenetic/molecular unresponsiveness or (ii) detection of increasing percentages of Philadephia-chromosome positive metaphases and/or *BCR-ABL* positive interphase nuclei in FISH analysis, or (iii) an increase of the 100×*BCR-ABL/ABL* ratio of at least one log in two consecutive samples from CML patients receiving ongoing imatinib therapy.

### Statistics

The SPSS 12.0 for Windows software package (SPSS, Chicago, IL, USA) was used for statistical analysis. Fisher´s exact test was performed when applicable to analyze differences between cohorts of patients. Differences were statistically significant when two-sided $p$ values were less than 0.05. Using Kaplan-Meier estimates, curves were calculated for the probability of second relapse, overall survival, and leukemia-free survival from the start of therapy for the first relapse after allogeneic HSCT. The curves were compared using a double sided log rank test. Results were statistically significant at a level of $p<0.05$ at both sides.

## Results and Discussion

### Best response to therapy

After DLI therapy 20/21 patients (95%) achieved a complete molecular remission. Imatinib therapy resulted in hematologic, complete molecular cytogenetic, and complete molecular genetic remission in 9/10 (90%), 7/10 (70%), and 7/10 (70%) patients, respectively. There was a trend towards a higher rate of molecular remissions assessed by real time RT-PCR for the DLI group ($p$=0.087). The time to achieve molecular complete remission was 7.5 months in the DLI group and 6 months in the imatinib group ($p$=1.0). *BCR-ABL* was undetectable by qualitative nested RT-PCR in 81% (17/21) of the DLI group and 50% (5/10) of the imatinib group ($p$=0.115 ).

### Relapse analysis

Among the ten patients treated with imatinib, relapse occurred in six (60%) patients while they were receiving imatinib therapy. In four patients imatinib was discontinued after achievement of complete molecular remissions. These four patients had received imatinib for at least 1 year (n=2), 2 years (n=1) or 3 years (n=1). Complete molecular remission had been ascertained in at least two consecutive samples from all of these partients before imatinib was discontinued. Only one of these four patients has remained in molecular remission, now lasting 2 years, after withdrawal of imatinib. The other three patients relapsed 2 (n=1) and 4 (n=2) months after cessation of imatinib. For therapy of second relapse imatinib was restarted in two patients, resulting in a complete molecular cytogenetic remission. The other seven patients received DLI, resulting in a complete molecular cytogenetic remission in six of them. One patient was refractory to DLI and underwent a second HSCT after which a complete molecular remission was achieved. Three patients (14%) relapsed after DLI. Two of these patients relapsed after achievement of a molecular remission. The other patient showed no molecular cytogenetic or molecular genetic response to DLI. The probability of relapse was signif-

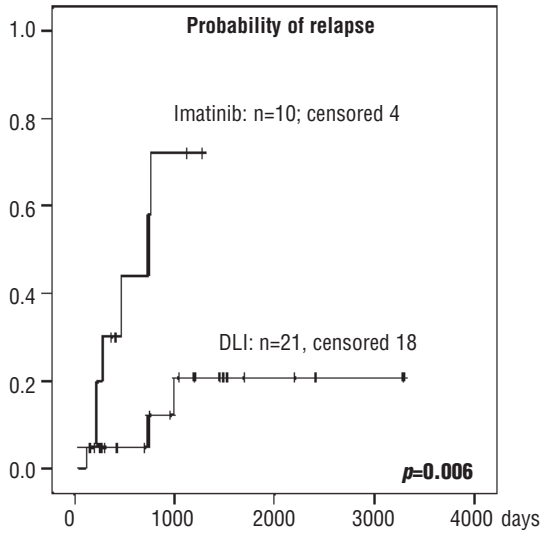


Figure 1. Probabilities of relapse in the imatinib group (n=10) and the DLI group (n=21; $p$=0.006). The patients in whom imatinib was discontinued were censored at the time of imatinib cessation.

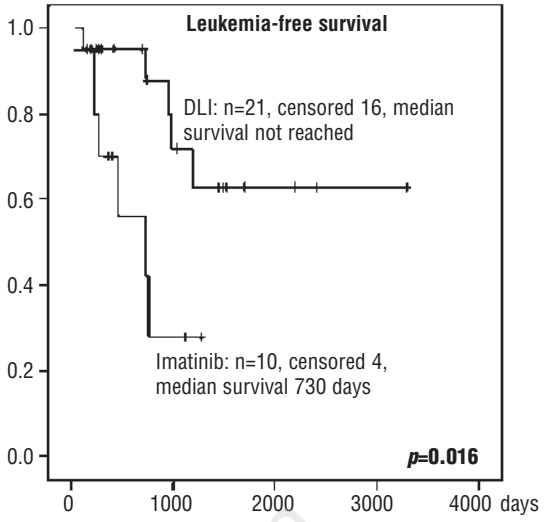


Figure 2. Kaplan-Meier plot of leukemia-free survival in the imatinib group (n=10) and the DLI group (n=21; $p$=0.016). The patients in whom imatinib was discontinued were censored at the time of imatinib cessation.

icantly higher in the imatinib group than in the DLI group ($p$=0.006; Figure 1).

### GvHD

GvHD grades II-IV requiring immunosuppressive therapy occurred in 11/21 (52%) patients after DLI therapy. Seven patients developed extensive chronic GvHD. Resolution of GvHD was observed in four patients. No GvHD reaction was seen after imatinib therapy.

### Leukemia-free survival and overall survival

The rate of leukemia-free survival was significantly higher in the DLI group than in the imatinib group ($p$=0.016; Figure 2). Three patients died in the DLI group, two of GvHD and one of progressive leukemia. The rate of treatment-related mortality was, therefore, 10% in the DLI group whereas there were no treatment-related deaths in the imatinib group ($p$=0.533). The probability of overall survival at 5 years was 100% in the imatinib group and 76% in the DLI group ($p$=0.183). We retrospectively compared the long-term outcome of patients treated with imatinib or DLI for relapse after allogeneic HSCT. There was a trend towards higher rates of complete molecular remissions in the DLI-treated group. Among the ten patients treated with imatinib six (60%) relapsed under ongoing imatinib therapy. Another three patients relapsed after discontinuation of imatinib. Only one patient remained in molecular remission after imatinib withdrawal. This observation suggests that despite molecular responses, documented over 2 years at longest, it is unlikely that patients can be cured of CML by imatinib alone and that cellular therapy for relapse after HSCT is inevitable. This observation is in line with a report by Cortes and co-workers demonstrating rapid relapse in conventionally treated CML patients after discontinuation of imatinib.[1,2] The higher relapse rate in the imatinib group also resulted in an inferior leukemia-free survival in these patients as compared to those treated with DLI. Studies on the treatment of relapse with DLI have previously shown that DLI from unrelated donors are associated with lower response rates[1] and a higher risk of treatment-related mortality.[3] Thus, although the differences in donor distribution of the present study were not statistically significant they may have influenced the outcome in favor of the DLI group. Acute GvHD was observed in more than half of the patients treated with DLI and led to extensive chronic GvHD in one third of the patients. In total, three patients died after DLI – two of GvHD, and one of progressive disease. The rate of treatment-related mortality was 10% in the DLI group and 0% in the imatinib group. Thus there was a trend towards a better overall survival in the imatinib group. However, overall survival figures should be interpreted cautiously as the follow-up was shorter in the imatinib group and, considering the high incidence of relapse, patients will subsequently receive DLI as a second treatment. Taking into account the limitations of the low numbers of patients studied, our data suggest that imatinib, in contrast to DLI, does not provide definite cure for relapsed CML after allogeneic HSCT. These observations need to be confirmed in more patients in prospective trials.

*MW: data analysis, wrote the manuscript; JT: data analysis; SS: molecular genetic analysis; CS: cytogenetic analysis; GL: data analysis; HJK: Head of BMT-Unit, responsible author. The authors declare that they have no potential conflicts of interest. Manuscript received October 25, 2005. Accepted March 6, 2006.*

## References


1. Guglielmi C, Arcese W, Dazzi F, Brand R, Bunjes D, Verdonck LF, et al. Donor lymphocyte infusion for relapsed chronic myelogenous leukemia: prognostic relevance of the initial cell dose. Blood 2002;100:397-405.
2. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. European Group for Blood and Marrow Transplantation Working Party Chronic Leukemia. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86:2041-50.
3. Raiola AM, Van Lint MT, Valbonesi M, Lamparelli T, Gualandi F, Occhini D, et al. Factors predicting response and graft-versus-host disease after donor lymphocyte infusions: a study on 593 infusions. Bone Marrow Transplant 2003;31:687-93.
4. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001; 344:1031-7.
5. O'Brien SG, Guilhot F, Larson RA, Gathmann I, Baccarani M, Cervantes F, et al. Imatinib compared with interferon and low-dose cytarabine for newly diagnosed chronic-phase chronic myeloid leukemia. N Engl J Med 2003; 348: 994-1004.
6. Ullmann AJ, Hess G, Kolbe K, Friedrich-Freksa A, Meyer RG, Gschaidmeier H, et al. Current results on the use of imatinib mesylate in patients with relapsed Philadelphia chromosome positive leukemia after allogeneic or syngeneic hematopoietic stem cell transplantation. Keio J Med 2003;52: 182-8.
7. DeAngelo DJ, Hochberg EP, Alyea EP, Longtine J, Lee S, Galinsky I, et al. Extended follow-up of patients treated with imatinib mesylate (Gleevec) for chronic myelogenous leukemia relapse after allogeneic transplantation: durable cytogenetic remission and conversion to complete donor chimerism without graft-versus-host disease. Clin Cancer Res 2004;10:5065-71.
8. Kantarjian HM, O'Brien S, Cortes JE, Giralt SA, Rios MB, Shan J, Giles FJ, et al. Imatinib mesylate therapy for relapse after allogeneic stem cell transplantation for chronic myelogenous leukemia. Blood 2002;100:1590-5.
9. Schoch C, Schnittger S, Bursch S, Gerstner D, Hochhaus A, Berger U, et al. Comparison of chromosome banding analysis, interphase- and hypermetaphase-FISH, qualitative and quantitative PCR for diagnosis and for follow-up in chronic myeloid leukemia: a study on 350 cases. Leukemia 2002; 16: 53-9.
10. Emig M, Saussele S, Wittor H, Weisser A, Reiter A, Willer A, et al. Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real time quantitative RT-PCR. Leukemia 1999;13:1825-32.
11. Maurer J, Janssen JW, Thiel E, van Denderen J, Ludwig WD, Aydemir U, et al. Detection of chimeric BCR-ABL genes in acute lymphoblastic leukaemia by polymerase chain reaction. Lancet 1991;337:1055-8.
12. Cortes J, O'Brien S, Kantarjian H. Discontinuation of imatinib therapy after achieving a molecular response. Blood 2004;104:2204-5.